

THE ACCESSORY CHROMOSOME OF ANASA TRISTIS AGAIN

By

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During the examination of literature in connection with cytological studies on other Hemiptera I became much interested in the case of *Anasa tristis*. I was astonished to find a very marked variation in the results of the cytologists who have studied the spermatogenesis of this form. Therefore, when in the summer of 1919 there occurred an excellent opportunity to procure an abundance of material, I decided to make some observations of an independent nature. It would seem not out of place occasionally to examine some of the commonly accepted cases and particularly where, as in this instance there has been a decided disagreement.

Altogether ten investigators have worked on the male germ-cells of *Anasa tristis*. At one time or another four of this number have been opposed to the now generally accepted view, first stated by E. B. Wilson. Two of these have corrected their former statements and now agree with the latter in his conclusions.

Paulmier ('99), who was the next after Henking ('91) to study the history of the accessory chromosome, decided that the spermatogonial number of chromosomes was twenty-two. He discovered the pair of m-chromosomes in the spermatogonia and described their behavior. He believed these united in synapsis to form a single condensed bivalent chromosome-nucleolus which persisted throughout the growth period and became the small central tetrad of the first maturation division. Furthermore he stated that this tetrad divided equally in the first division but that the products of this division passed undivided to but one of the poles of the second spindle giving ten and eleven chromosomes respectively to the spermatids. He therefore identified the chromosome-nucleolus of the growth period as the microchromosome bivalent and thought this to be identical with the accessory.

Montgomery ('01, '04) followed Paulmier in giving the spermatogonial number of chromosomes as twenty-two. He also regarded the accessory chromosome as being derived by a fusion of the m-chromosomes. A re-examination of his material after the publication of Wilson's second paper on chromosomes led to a change in his statements so that they were then in agreement.

After a very careful study of Paulmier's material as well as his own Wilson came to the following conclusions in a remarkable series of papers ('05, '06, '07, '11); (1) that there are twenty-one and not twenty-two

chromosomes in the spermatogonia; (2) that these chromosomes exist in pairs and this leaves one without a mate; (3) that this odd one is one of the three largest and is the so-called accessory chromosome; (4) that this chromosome exists as a chromosome-nucleolus throughout the growth period; (5) that the m-chromosomes previously identified by other observers as the accessory have an entirely independent history and divide in both of the maturation divisions; (6) but that the real accessory divides in the first and then passes undivided to one only of the two spermatids derived by division of the secondary spermatocytes, thus giving rise to two kinds of spermatozoa.

Foot and Strobell ('07), using smear methods to the entire exclusion of sections and illustrating only with photo-micrographs, took sharp issue with Wilson. These investigators asserted that Paulmier was right in his spermatogonial count; that the so-called chromosome-nucleolus of the growth period is but "morphologically the equivalent of a nucleolus" or in other words the plasmosome; that there is no odd or accessory chromosome; that what has been called such is but a lagging chromosome which divides in each division as do all the others; that therefore all spermatids receive eleven chromosomes.

This disagreement among cytologists, of course, became a serious matter. Many fundamental facts came into direct question and consequently several people were interested enough to make independent investigation of the conditions.

Closely following the papers of Foot and Strobell there appeared a brief treatment of the question by Lefevre and McGill ('08). Their observations confirmed those of Wilson.

In connection with work on the chromosomes of some of the coreid Hemiptera Morrill ('10) confirmed Wilson's spermatogonial count.

The climax of the researches on the accessory chromosome of *Anasa* came in 1910 when McClung and Pinney went over the whole matter with great care. Miss Pinney made an entirely independent study and in order to avoid bias or prejudice in the matter refrained from reading any of the accounts published by other investigators until her own conclusions had been reached. McClung studied the original material of Paulmier, Wilson and Lefevre and McGill. Both McClung and Pinney agreed that the spermatogonial number is twenty-one. They further agreed with Wilson that there are ten bivalent chromosome and one, the accessory, which is univalent in the metaphase of the first division. This univalent body exists as a short, heavy thread, a compact mass or finally as a straight longitudinally split rod all through the prophase stages of the first division. It divides as do the others in the first but does not divide in the second mitosis.

In making this brief and confirmatory study of *Anasa tristis*¹ I have used the ordinary cytological methods now somewhat standardized. As usual I have found Bouin's and Flemming's fluids very valuable. Perhaps the best preparations have been made with Bouin's. The iron-hæmatoxylin method of staining has again proven the best general stain although I have had difficulty with the domestic preparations.

My own conclusions are not enough different from those of Wilson to warrant a lengthy treatment. In fact as regards the important stages the matter might be dismissed by a statement that the facts as I see them are as stated by Wilson, Lefevre and McGill, and McClung and Pinney. In order, however, that my results may be on record and that the constancy of the chromosome relationship within the species may be further evidenced I will state the main facts as I see them.

There are without question twenty-one chromosomes in the normal spermatogonia. I have examined dozens of these cells in the metaphase when the chromosomes are well spread out but I have never found one which clearly showed more than the expected number. The three large bean-shaped chromosomes and two small m-chromosomes are always present together with sixteen others of about equal size.

In the late prophase stages of the first division at least nine typical tetrads can be seen accompanied by what I think are three dyad bodies. I am sure one of these is the accessory. Probably the others are the m-chromosomes but the study of these has not made me entirely sure of this. When the chromosomes become placed in the metaphase plate preparatory to division the accessory usually occupies a position outside of the ring formed by the position of the nine ordinary tetrads. The m-chromosomes, now unquestionably formed into a tetrad, occupy a central position within the ring. All the bodies divide equally in this division. As a result then all secondary spermatocytes possess eleven chromosomes each. When these become arranged in the flat metaphase plate again the ring-like arrangement is succeeded by an irregular placing. In spite of this the accessory can usually be identified. When the second division occurs this accessory does not divide like the rest but goes to one pole undivided thus giving rise to two kinds of spermatids. Lateral and polar views of these stages show without the shadow of doubt that half the spermatids receive ten while the other half get eleven chromosomes.

At this point I will mention a condition observed several times in *Anasa* and other Hemiptera. Follicle cells in division frequently show double the normal spermatogonial number of chromosomes. How this is brought about and what the fate of the cells involved is I am not able to state.

¹ For the identification of my material I am indebted to Dr. Paul S. Welch of the Univ. of Michigan, Ann Arbor, Mich.

It is amazing that there should have been so much disagreement in the results of investigations up to this point. As is often the case in the Hemiptera when the preparations are good most of the stages so far outlined stand out with diagrammatic clearness. There should be no further difference of opinion in regard to these matters. Anyone can demonstrate the truth of the statements by using reasonable care in preparation and observation.

When we come to the question of the behavior of the accessory chromosome from the time of the last spermatogonial division down to the late prophases of the first maturation division the facts are more difficult to determine. Those who have worked upon the question of spermatogenesis will agree that it is often difficult to discover just what is going on in the nucleus at this time. However, I believe the main contentions of Wilson can be proven true

In many cells before and during synezesis a compact body can be found just outside of the more or less tangled mass of chromatin threads. Because of its size and appearance I think it reasonable to conclude that it is the accessory chromosome. In every stage following this the same body can be identified and need not be confused with the plasmosome which occurs with it for a large part of the growth period. Thus the accessory maintains its individuality throughout the maturation process.

In conclusion I would say then that I believe the current explanation of the "case of *Anasa tristis*" as given by text-books of zoology and genetics based upon the work of Wilson is correct. This classical example may be regarded as a permanent addition to our stock of knowledge. Our interpretations may change, of course, but the facts will stand as Wilson stated them.

The different stages of the spermatogenesis of *Anasa* are on the whole so clear and beautiful that I can recommend them for class use where it is desired to demonstrate the main facts connected with the behavior of the sex chromosome.

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